

AMENDMENTS TO THE CLAIMS

This listing of claims replaces all previous versions, and listings, of claims pending in this application.

Listing of Claims

1. **(Withdrawn)** A method of polynucleotide synthesis, comprising: combining in a polymerization reaction mixture a thermostable polymerase, a template nucleic acid molecule, appropriate primers for the template nucleic acid molecule, at least one deoxynucleoside triphosphate, and a non-nucleic acid polyanion, wherein the temperature of the polymerization reaction mixture is at a temperature at which the non-nucleic acid polyanion inhibits thermostable polymerase activity; and heating the polymerization reaction mixture to a temperature at which the non-nucleic acid polyanion dissociates from the thermostable polymerase, allowing the thermostable polymerase to recognize and provide polynucleotide synthesis on a primer annealed nucleic acid molecule.
2. **(Withdrawn)** The method of claim 1 wherein the polynucleotide synthesis is polymerase chain reaction
3. **(Withdrawn)** The method of claim 1 wherein the non-nucleic acid polyanion has a molecular weight of from 1500 to 500,000.
4. **(Withdrawn)** The method of claim 1 wherein the non-nucleic acid polyanion has a molecular weight of from 4,000 to 15,000.
5. **(Withdrawn)** The method of claim 1 wherein the non-nucleic acid polyanion has a molecular weight of from 5,000 to 10,000.
6. **(Withdrawn)** The method of claim 1 wherein the non-nucleic-acid polyanion is a synthetic organic polysulfate selected from the group poly(anetholsulfonic acid) polyvinyl sulfate and polystyrene sulfate.
7. **(Withdrawn)** The method of claim 6 wherein the non-nucleic acid polyanion is a sulfated oligo- or polysaccharide.
8. **(Withdrawn)** A method of polynucleotide synthesis, comprising: combining in a polymerization

reaction mixture a thermostable polymerase, a template nucleic acid molecule, appropriate primers for the template nucleic acid molecule, at least one deoxynucleoside triphosphate, and a polymer or copolymer of sugars selected from the group consisting of glucose, N-acetyl-glucosamine, galactouronic acid, hyalouronic acid, Nacetyl-galactosamine and sulfated fucose, wherein the temperature of the polymerization reaction mixture is at a temperature at which the polymer or copolymer inhibits thermostable polymerase activity; heating the polymerization mixture to a temperature at which the template nucleic acid molecule is denatured from a double-stranded molecule to a single-stranded molecule; cooling the polymerization mixture to a temperature of from about 45° C to about 65° C to allow appropriate primers to anneal to the single-stranded molecule; and modifying the polymerization mixture to a temperature at which the polymer or copolymer is substantially dissociated from the thermostable polymerase and the thermostable polymerase recognizes and provides polynucleotide synthesis on primer annealed nucleic acid molecule.

9. **(Withdrawn)** The method of claim 8 wherein the sulfated polymer or copolymer of sugars is selected from the group consisting of dextran sulfate, fucoidan, heparin; heparan sulfate, chondroitin polysulfate, keratan polysulfate, xylaR poly, sulfate, and pentosan polysulfate.

10. **(Withdrawn)** The method of claim 1 wherein the non-nucleic acid polyanion is at a final reaction concentration of from 0.1 μ M to 1.5 μ M.

11. **(Withdrawn)** The method of claim 1 wherein the non-nucleic acid polyanion is at a final reaction concentration of from 0.2 μ M to 1.0 μ M.

12. **(Withdrawn)** A method of polynucleotide synthesis, comprising: combining in a polymerization reaction mixture a thermostable polymerase, a template nucleic acid molecule, appropriate primers for the template nucleic acid molecule, at least one deoxynucleoside triphosphate, and a non-nucleic acid polyanion, wherein the temperature of the polymerization reaction mixture is at a temperature at which the non-nucleic acid polyanion inhibits thermostable polymerase activity; heating the polymerization reaction mixture to a temperature at which the template nucleic acid molecule is denatured from a double-stranded molecule to a single-stranded molecule; cooling the polymerization reaction mixture to a temperature at which appropriate primers anneal to the single-stranded molecule; and modifying the temperature of the polymerization reaction mixture to 60° C to 75° C wherein the non-nucleic polyanion substantially ceases to inhibit thermostable polymerase activity.

13. **(Withdrawn)** A method of polynucleotide synthesis, comprising: combining in a polymerization reaction mixture a thermostable polymerase selected from the group consisting of DNA polymerase, RNA polymerase, reverse transcriptase, and mixtures thereof, a template nucleic acid molecule, and appropriate primers for the template nucleic acid molecule, at least one deoxynucleoside triphosphate, and a non-nucleic acid polyanion, wherein the temperature of the polymerization reaction mixture is at a temperature at which the non-nucleic acid polyanion inhibits thermostable polymerase activity; heating the polymerization reaction mixture to a temperature at which the template nucleic acid molecule is denatured from a double-stranded molecule to a single-stranded molecule; cooling the polymerization reaction mixture to a temperature at which appropriate primers anneal to the single-stranded molecule; and modifying the temperature of the polymerization reaction mixture to a temperature at which the non-nucleic acid polyanion is substantially dissociated from the thermostable polymerase, wherein the thermostable polymerase recognizes and provides polynucleotide synthesis on primer annealed nucleic acid molecule.

14. **(Withdrawn)** The method of claim 13 wherein the reverse transcriptase is a derivative, mutant or chimeric complex of the reverse transcriptase.

15. **(Original)** A kit for polynucleotide synthesis on a target nucleic acid, the kit comprising: a thermostable polymerase reversibly bound to a non-nucleic acid polyanion; and an appropriate polymerase reaction buffer.

16. **(Original)** The kit of claim 15 wherein the thermostable polymerase is *Thermus aquaticus*.

17. **(Original)** The kit of claim 15 wherein the non-nucleic acid polyanion is dextran sulfate.

18. **(Original)** The kit of claim 15 further comprising at least one nucleotide 5'-triphosphate.

19. **(Original)** The kit of claim 15 further comprising a pair of primers for the target nucleic acid.

20. **(Original)** The kit of claim 15 wherein the non-nucleic acid polyanion has a molecular weight of from 1,500 to 500,000.

21. **(Original)** The kit of claim 15 wherein the non-nucleic acid polyanion has a molecular weight of from 4,000 to 15,000.

22. **(Original)** A composition for polynucleotide synthesis comprising: a thermostable polymerase; a non-nucleic acid polyanion; a polymerase reaction buffer having monovalent cations between 35-60 mM; at least one dNTP; a template nucleic acid molecule; and appropriate template nucleic acid primers.

23. **(Original)** The composition of claim 22 wherein the non-nucleic acid polyanion has a molecular weight of from 1,500 to 500,000.

24. **(Original)** The composition of claim 22 wherein the non-nucleic acid polyanion has a molecular weight of from 4,000 to 15,000.

25. **(Original)** The composition of claim 22 wherein the non-nucleic acid polyanion has a molecular weight of from 4,000 to 10,000.

26. **(Original)** The composition of claim 22 wherein the non-nucleic acid polyanion is a synthetic organic polysulfate selected from the group poly(anetholsulfonic acid), polyvinyl sulfate, and 15 polystyrene sulfate.

27. **(Original)** The composition of claim 26 wherein the anionic polysulfate is a sulfated oligo- or polysaccharide.

28. **(Original)** The composition of claim 27 wherein the sulfated oligo- or polysaccharide is a sulfated polymer or copolymer of the sugars selected from the group consisting essentially of glucose, N-acetyl-glucosamine, galactouronic acid, hyalouronic acid, N-acetyl-galactosamine and fucose.

29. **(Original)** The composition of claim 28 wherein the sulfated polymer or copolymer of the sugar is selected from the group consisting essentially of dextran sulfate, fucoidan, heparin, heparan sulfate, chondroitin polysulfate, keratan polysulfate, xylan polysulfate, and pentosan polysulfate.

30. **(Original)** The composition of claim 22 wherein the non-nucleic acid polyanion is at a concentration of from 0.1 μ M to 1.5 μ M.

31. **(Original)** The composition of claim 22 wherein the non-nucleic acid polyanion is at a concentration of from 0.2 μ M to 1.0 μ M.

32. **(Original)** The composition of claim 22 wherein the thermostable polymerase is selected from the group consisting essentially of DNA polymerase, RNA polymerase, reverse transcriptase, and mixtures thereof.

33. **(Original)** The composition of claim 32 wherein the thermostable polymerase is a DNA polymerase and the DNA polymerase is from a thermophilic Eubacteria or a Archaeobacteria.

34. **(Original)** The composition of claim 33 wherein the thermostable polymerase is selected from the group consisting essentially of *Thermus aquaticus*, *T. thermophilus*, *T. Brockianus*, *T. flavus*, *T. ruber*, *Thermatoga maritima*, *Thermoplasma acidophilus*, *Pyrococcus furiosus*, *Pyrococcus woessii*, *Pyrococcus spec.*, *Sulfolobus spec.*, and mixtures thereof.

35. **(Original)** The composition of claim 32 wherein the thermostable polymerase is a reverse transcriptase and wherein the reverse transcriptase is selected from the group consisting essentially of MmLV reverse transcriptase, AMV reverse transcriptase, RSV reverse transcriptase, HIV-1 reverse transcriptase, HIV-2 reverse transcriptase, and mixtures thereof.

36. **(Withdrawn)** The method of claim 12 wherein the non-nucleic acid polyanion has a molecular weight of from 4,000 to 15,000.

37. **(Withdrawn)** The method of claim 12 wherein the non-nucleic acid polyanion has a molecular weight of from 4,000 to 10,000.

38. **(Withdrawn)** The method of claim 8 wherein the modifying of the polymerization mixture to a temperature at which the non-nucleic acid polyanion is substantially dissociated from the thermostable polymerase is from 60° C to 75° C.

39. **(New)** A method of polynucleotide synthesis, comprising: combining a kit according to claim 15 with a polymerization reaction mixture comprising a target nucleic acid and appropriate primers, wherein the temperature of the polymerization reaction mixture is at a temperature at which the non-nucleic acid polyanion inhibits thermostable polymerase activity; and heating the polymerization reaction mixture to a temperature at which the non-nucleic acid polyanion dissociates from the thermostable polymerase, thereby permitting elongation of the target nucleic acid.

Serial No.: 10/661,428
Filing Date: September 11, 2003

40. (New) A method of polynucleotide synthesis, comprising: preparing a polymerization reaction mixture comprising the composition according to claim 32, a template nucleic acid molecule, and appropriate primers for the template nucleic acid molecule, wherein the temperature of the polymerization reaction mixture is at a temperature at which the non-nucleic acid polyanion inhibits thermostable polymerase activity; and heating the polymerization reaction mixture to a temperature at which the non-nucleic polyanion dissociates from the thermostable polymerase, thereby permitting elongation of the template nucleic acid molecule..

41. (New) The method of claim 39, wherein the polymerization reaction mixture is heated to a temperature of 60° C to 75° C.

42. (New) The method of claim 40, wherein the polymerization reaction mixture is heated to a temperature of 60° C to 75° C.